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THE GROWTH OF RHODOSPIRILLUM RUBRUM AND PHOTOBACTERIUM  
FISCHERI IN MIXED CULTURE

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by  
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## CHAPTER I

### INTRODUCTION

Growth of Paramecium aurelia and Paramecium caudatum in the same microcosm with a limited energy source, has been demonstrated by Gause, Nastukova and Alpatov. It has been determined that the alteration in the growth of one or both species will occur when the two are grown together in a mixed culture.<sup>1</sup>

It would be interesting to determine the effects produced when photosynthetic organisms are grown in the presence of bioluminescent organisms. The bioluminescent bacteria Photobacterium fischeri and the photosynthetic bacteria Rhodospirillum rubrum appear to be well-adapted for this kind of study. The bacteria can be grown both in total darkness and in constant light, as well as in pure and mixed culture.

It has been shown by Dubois that Photobacterium fischeri emits light when grown aerobically in a seawater environment containing a concentration of two to three per cent sodium chloride.<sup>2</sup> Rhodospirillum rubrum will grow

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<sup>1</sup>G. F. Gause, The Struggle for Existence (Baltimore: The Williams and Wilkins Company, 1934), pp. 98-113.

<sup>2</sup>Raymond Dubois, "Physiological Light," Smithsonian Report, (July, 1896), 413-431.

anaerobically in the presence of light under reduced carbon dioxide tension and aerobically in the presence of light.<sup>1</sup>

It is, therefore, the purpose of this investigation to study the growth of Rhodospirillum rubrum and Photobacterium fischeri in a mixed culture under constant light and darkness.

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<sup>1</sup>Howard Gest and M. D. Kamen, "Photoproduction of Molecular Hydrogen in Rhodospirillum Rubrum," Science, CIX (June, 1949), 559.

## CHAPTER II

### HISTORY

Population studies of mixed species are usually concerned with competition for food, niche, or space. It has been found there are four biological possibilities which may occur when species of organisms are mixed.

1. Each species inhibits its own potential increase more than that of the other and both continue to exist together.
2. The second species inhibits the potential increase of the first less than it inhibits its own, while the first species inhibits the potential increase of the second more than it inhibits its own, whatever the initial number of the two species; the first species drives out the second.
3. This is the opposite to two and the second species drives out the first.
4. Each species inhibits the other more than itself; one drives out the other depending chiefly on the initial sizes of their populations.<sup>1</sup>

#### I. PHOTOBACTERIUM FISCHERI

Bioluminescent bacteria and other luminescent organisms have been described by man since early recorded history. It was not until the advent of the microscope and the discovery of bacteria that a comprehensive study of bioluminescent bacteria was begun.

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<sup>1</sup>W. C. Allee and others, Principles of Animal Ecology (Philadelphia: W. B. Saunders Company, 1949), p. 369.



Raphael Dubois in 1895 reported on the morphology of the Photobacteriaceae. He found that the Photobacterium were usually elongated; their length varying between two to four microns and their width one to two microns. Dubois distinguished seven species of these bacteria. Some were very polymorphous and changed into micrococci, spiral or bacilli forms without ceasing to be luminous. At other times, even though the form remained unchanged, the photogenic function could be made to disappear by modifying the culture slightly.

Dubois reported that organisms could be cultured in four per cent bouillon of gelatin, peptone and sea water. Dubois found that Photobacterium sacochilum developed well in nutrient broth. This was the first photogenic bacteria which was successfully cultured in a chemically defined liquid medium containing water, glycerin, phosphates asparagin and sea salt.

The first definitive experiment regarding the nature of the components necessary for light production were also carried out by Dubois. Dubois proposed the theory that two substances were necessary for light production, one which he called luciferin, and another substance which he called luciferase which was unstable to heat.<sup>1</sup>

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<sup>1</sup>Raphael Dubois, "Physiological Light," Smithsonian Report, (July, 1895), 413-431.

In the early 1930's Irvin M. Korr tried to demonstrate the luciferin-luciferase reaction by using *Photobacterium*. The methods used by Korr to initiate the light reaction included (1) cytolysis by fat solvents, (2) osmotic cytolysis, (3) mechanical grinding, and (4) intense sonic vibration. Although all of these experiments were carried out under the same conditions which were in general favorable for bioluminescence, it was not possible in any of the cases to demonstrate the luciferin-luciferase reaction or obtain luminescence from bacteria whose structure had been materially altered. Korr therefore theorized that bioluminescence, like many other oxidative phenomena, was closely associated with cellular structure.<sup>1</sup>

In 1954 W. D. McElroy and others were able to successfully separate the light emitting substance from Achromobacter fischeri by acid precipitation and fractionating with ammonium sulphate. With the partially purified enzyme it was possible to show that an absolute requirement for light emission was reduced diphosphopyridine nucleotide and flavin mononucleotide. In addition to these two factors a third component was found which was activated by ultra violet

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<sup>1</sup>Irvin M. Korr, "The Relation Between Cell Integrity and Bacterial Luminescence," Biological Bulletin, LXVIII (January, 1935), 61-62.

light. This factor was classified as bacterial luciferin.<sup>1</sup>

Other investigations into the light reaction of Achromobacter fischeri has shown further details necessary for the production of light. Some of these are as follows: (1) The light emitted depends upon a series of electron transfer reactions. (2) The essential components of the light production are reduced flavin mononucleotide (FMNH<sub>2</sub>), a higher fatty aldehyde from C<sub>6</sub>-C<sub>16</sub>, atmospheric oxygen, and an extract of bacterial enzymes. (3) Substrate and phosphopyridine nucleotides (DPN, TPNH) or reduced redox substances with potentials more negative than -81 mv can serve as electron sources for the reduction of flavin mononucleotide (FMN).<sup>2</sup>

Investigation by Farghaly in 1950 has demonstrated that luminous bacteria are normally grown in salt concentrations between 2.5 and 3 per cent sodium chloride. By lowering the salt concentration below 2.5 per cent, growth becomes increasingly proliferative, while raising the salt concentration up to 3 per cent yields an increase in

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<sup>1</sup>W. D. McElroy and others, "Partial Purification and Properties of Bacterial Luciferin and Luciferase," Journal of Bacteriology, LXVIII (February, 1954), 130.

<sup>2</sup>Fred E. Hahn, "Action of Selected Redox Substances Bacterial Bioluminescences," Science, CXXVII (January, 1958), 188-189.

luminescence.<sup>1</sup>

## II. RHODOSPIRILLUM RUBRUM

Rhodospirillum rubrum may grow in the presence of or in the absence of light. In the presence of light and in the presence of synthetic medium consisting of pure organic substrates, mineral salts including ammonium chloride and a trace of biotin, Rhodospirillum rubrum carries on photosynthesis and anaerobic metabolism.<sup>2</sup>

Kohmiller and Gest have found anaerobic decomposition of pyruvic acid in the dark by resting cells to be a typical propionic acid fermentation. Cells grown aerobically in the dark also ferment pyruvate with production of carbon dioxide, small quantities of hydrogen, and acetic, propionic, butyric, valeric and caproic acid. Whether or not aerobic or anaerobic respiration is to be carried out depends on the carbon dioxide tension.

Light induced decomposition of pyruvate is characterized by the production of approximately equimolar quantities of carbon dioxide and hydrogen and the absence of fatty acids

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<sup>1</sup>A. H. Farghaly, "Factors Influencing the Growth and Light Production of Luminous Bacteria," Journal of Cellular and Comparative Physiology, XXXVI (August, 1950), 165-183.

<sup>2</sup>Howard Gest and Martin D. Kamen, "Photoproduction of Molecular Hydrogen in Rhodospirillum Rubrum," Science, CIX (June, 1949), 559.

as an end product. After a lag period dark-grown cells exposed to light will ferment pyruvate in a similar manner.<sup>1</sup>

Rhodospirillum rubrum differs from the non-sulfur bacteria in two major respects: (1) For growth, Rhodospirillum rubrum requires one or more preformed vitamins. (2) Certain strains of Rhodospirillum rubrum are capable of multiplying in the dark under the proper conditions.<sup>2</sup>

Hickman and Frenkel have found that Rhodospirillum rubrum possesses a multilayered outer envelope, and the external cell surface is differentiated into ridges extending parallel or obliquely to the long axis of the cell. The organisms from young cultures resemble non-photosynthetic bacteria and contain only a granular cytoplasm. They contain neither chromatophores nor lamellar systems assumed by previous investigators to be characteristic of Rhodospirillum rubrum when grown anaerobically in the light. It was found that chromatophores appear in cells from cultures older than twelve hours, and paired lamella appeared along with the chromatophores in cells from cultures older than eight days.<sup>3</sup>

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<sup>1</sup>Elmer F. Kohmiller and Howard Gest, "A Comparative Study of Light and Dark Fermentation of Organic Acids by Rhodospirillum Rubrum," Journal of Bacteriology, LXI (March, 1951), 269.

<sup>2</sup>Howard Gest, "Metabolic Patterns in Photosynthetic Bacteria," Bacteriological Reviews, XV (March, 1953), 183.

<sup>3</sup>Donald D. Hickman and others, "The Structure of Rhodospirillum Rubrum," Journal of Biophysical and Biochemical Cytology, LXIV (August-December, 1959), 277-284.

Tuttle and Gest have suggested that the photoactive system of Rhodospirillum rubrum is associated with the cytoplasmic membrane and/or a reticulum of membranous extensions penetrating the cytoplasm. A corollary of this interpretation suggests that the pigment complex does not normally exist in the form of cytoplasmic inclusions comparable to the chloroplasts of higher plants.<sup>1</sup>

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<sup>1</sup>Alice L. Tuttle and H. Gest, "Subcellular Particulate Systems and the Photochemical Apparatus of Rhodospirillum rubrum," Proceedings National Academy of Science, XLV (July, 1959), 1261-1269.

## CHAPTER III

### METHODS AND MATERIALS

The bacterial organisms Photobacterium fischeri and Rhodospirillum rubrum were used in this investigation. The Photobacterium fischeri was obtained from a culture maintained by Drake University, while the Rhodospirillum rubrum culture was obtained from the American Type Culture Collection, Washington, D. C. Stock cultures of each organism were kept under refrigeration and transferred to new media every thirty days. Stock cultures of Photobacterium fischeri were maintained on Photobacterium agar slants (Difco). Rhodospirillum rubrum stock cultures were grown on a medium containing one per cent glucose, five tenths per cent yeast extract and one and one-half per cent bacto-agar (Difco).

Photobacterium broth, as listed below, was prepared and used for the growth of both organisms in the experimentals conducted in this investigation:

<u>Chemical</u>	<u>Amount</u>
Ammonium chloride	00.20 grams
Biotin	00.50 grams
Calcium carbonate	01.00 grams
Ferric chloride	00.01 grams
Magnesium sulphate	00.20 grams
Potassium dihydrogen phosphate	06.00 grams
Sodium chloride	20.00 grams
Sodium glycono-phosphate	23.50 grams
Tryptone	05.00 grams
Water	01.00 liter
Yeast Extract	05.00 grams

Growth studies both of Photobacterium fischeri and Rhodospirillum rubrum were carried out by culturing the organisms in three hundred milliliter Erlenmeyer flasks. Two hundred milliliters of Photobacterium broth medium was added to each flask, the flasks stoppered with cotton plugs, and autoclaved for twenty minutes at fifteen pounds pressure.

To prepare stock inocula of known age, each organism was grown separately in screw top, thirty-two milliliter culture tubes containing twenty milliliters of Photobacterium broth. Photobacterium fischeri was grown for a period of twenty hours before inoculating the experimental flasks. Rhodospirillum rubrum was grown for fifty hours before inoculation of the experimental flasks. After the growth period given above, one milliliter of the stock inocula was removed with a sterile pipette and discharged into each of the flasks containing two hundred milliliters of Photobacterium broth.

The growth of each experimental group was studied in triplicate. Each organism was first grown in pure culture and then in mixed culture. Growth of the mixed culture was started at such a time that both bacterial organisms began the logarithmic growth phase at the same time. In each case the culture was grown simultaneously in the dark and in constant light.

The pure and mixed cultures were inoculated in thermostat controlled paraffin ovens, (Precision Scientific Company)



at a constant temperature of twenty-five degrees centigrade. Each incubator was fitted with two doors which could be opened and closed. The inside door was composed of a glass plate and frame, while the outer door was composed of metal and lined with felt on the inside to insure darkness when closed.

For the growth of the organisms in light, the external door of the incubator was kept open while the inner glass door remained closed except for removing and replacing the bacterial cultures. A fifteen watt electric light bulb was suspended twenty-five centimeters from the cultures outside the incubator, and in this manner a constant light source was maintained throughout the experiment.

Organisms grown in darkness were kept in an identical incubator, but in total darkness. Darkness was insured by keeping the outer felt-lined door closed at all times, except when removing cultures for sampling. As further insurance of darkness, each three hundred milliliter flask was covered with aluminum foil and sealed with masking tape. The aluminum foil maintained darkness when the cultures were removed from the incubator for counting procedures.

Bacterial counts from pure and mixed cultures were made at intervals. A one milliliter sterile pipette was used for taking a one milliliter sample from each flask which was then placed into a twelve milliliter culture tube.

One-half milliliter of methyl violet (one milliliter saturated solution of methyl violet per one hundred milliliters of two per cent sodium chloride solution) was then added to each tube containing the one milliliter sample of bacteria. The tube containing the sample was then agitated and flamed lightly to insure fixation of the bacteria by the methyl violet stain.

A Petroff-Hausser and Halber Bacteria Counting Chamber, manufactured by C. A. Hausser and Son, Philadelphia, Pennsylvania, was used to determine bacterial counts. Bacterial samples were placed on the counting chamber between a cover glass and the counting field. Samples of bacteria were transferred from culture tubes to the counting chamber by use of capillary tubes, (seventy millimeters in length, a bore of five-tenths millimeter and external diameter of nine-tenths millimeter) obtained from Scientific Products Company, Evanston, Illinois.

A random sample of fifty squares on the counting chamber was counted for each bacterial reading. This was accomplished by recording all bacteria in the twenty squares on the horizontal to the right, then lowering the counting chamber two squares vertically and reading to the left. The last ten squares were then read by lowering two more squares vertically and reading ten squares to the right. An average number of bacteria per square was then determined. Each

square on the bacterial counting chamber was  $1/20$  mm x  $1/20$  mm x  $1/50$  mm depth or  $1/20,000$  cubic milliliters volume. The milliliter volume of each square was then corrected to one milliliter by multiplying by one thousand. A correction factor for the one-half milliliter of methyl violet stain was also used in determining the number of bacteria present in a one milliliter sample of culture.

To validate counting technique with the Petroff-Hausser counting chamber, bacterial counts were made using Photobacterium fischeri inoculated with serial dilutions of Photobacterium fischeri culture. It was determined that the growth pattern obtained by the pour plates counting technique was similar to that obtained by use of the Petroff-Hausser counting chamber.

Three counts were made from each bacterial sample. All bacterial counts were made using a Microstar Trinocular Microscope (Spencer), with a 10X ocular and a 100X oil immersion objective. A green filter (AO Spencer Ortho-Illuminator Model 600) was used to reduce glare and increase visibility of the bacteria. A counter manufactured by the Denominator Company, Incorporated, was used to record the number of bacteria observed during the counting process.

The Petroff-Hausser bacteria counter was cleaned after use with filter paper one-half inch by two and one-half inches in size.

The population of the mixed cultures was determined by first counting the number of Rhodospirillum rubrum present and then counting the number of Photobacterium fischeri present in a sample placed on the counting chamber.

## CHAPTER IV

### RESULTS AND INTERPRETATION OF DATA

The growth of Rhodospirillum rubrum and Photobacterium fischeri in mixed culture in constant light and dark is shown by the accompanying graphs and tables. The generation times and growth phases of each organism in constant dark and light in pure and mixed culture have been determined to show the growth pattern of the two bacterial species.

Cultures of both Rhodospirillum rubrum and Photobacterium fischeri when grown in pure and mixed culture in total light show negligible variation from pure and mixed cultures grown in total dark. Photobacterium fischeri will grow aerobically whether in the presence of light or dark. In the presence of light Rhodospirillum rubrum will carry on photosynthesis and anaerobic metabolism in the presence of a certain synthetic medium consisting of pure organic substrates and mixed salts, whereas aerobic metabolism is carried on in the absence of light. The medium which was used for the growth of Photobacterium fischeri was favorable for aerobic growth of Rhodospirillum rubrum, but anaerobic growth of Rhodospirillum rubrum in light was inhibited.

The growth patterns for Rhodospirillum rubrum and Photobacterium fischeri are described by Figures 1-4. Tables I and II show the generation times of each organism

respectively, while Tables III and IV show the lengths of the various growth phases. Growth phases of Photobacterium fischeri in pure and mixed culture and Rhodospirillum rubrum in pure culture were easily detected, since both organisms followed a typical sigmoid curve; however, the growth phases of Rhodospirillum rubrum when grown in mixed culture were not determined as easily, because Rhodospirillum rubrum growth was more variable.

The growth variation of Figures 1 and 2, pages 18 and 19, are described by Table I, page 22, which show the generation times of Rhodospirillum rubrum grown in pure and mixed culture in total dark and light. Hours twenty-five through thirty show a considerable variation in the generation times between the pure and mixed cultures. The generation time of the mixed culture was approximately one-third the length of time of the pure culture. The generation time for the pure culture was 4.13 hours, whereas the generation time of the mixed culture was 1.66 hours.

It can be seen that hours thirty through forty are the reverse of hours twenty-five to thirty. During hours thirty to forty the pure culture rate decreased its generation time to 1.29 hours, while the mixed culture increased in generation time to 2.83 hours. A continued decrease in generation time is seen from forty to fifty hours for the pure culture of 1.48 hours, while generation time for the

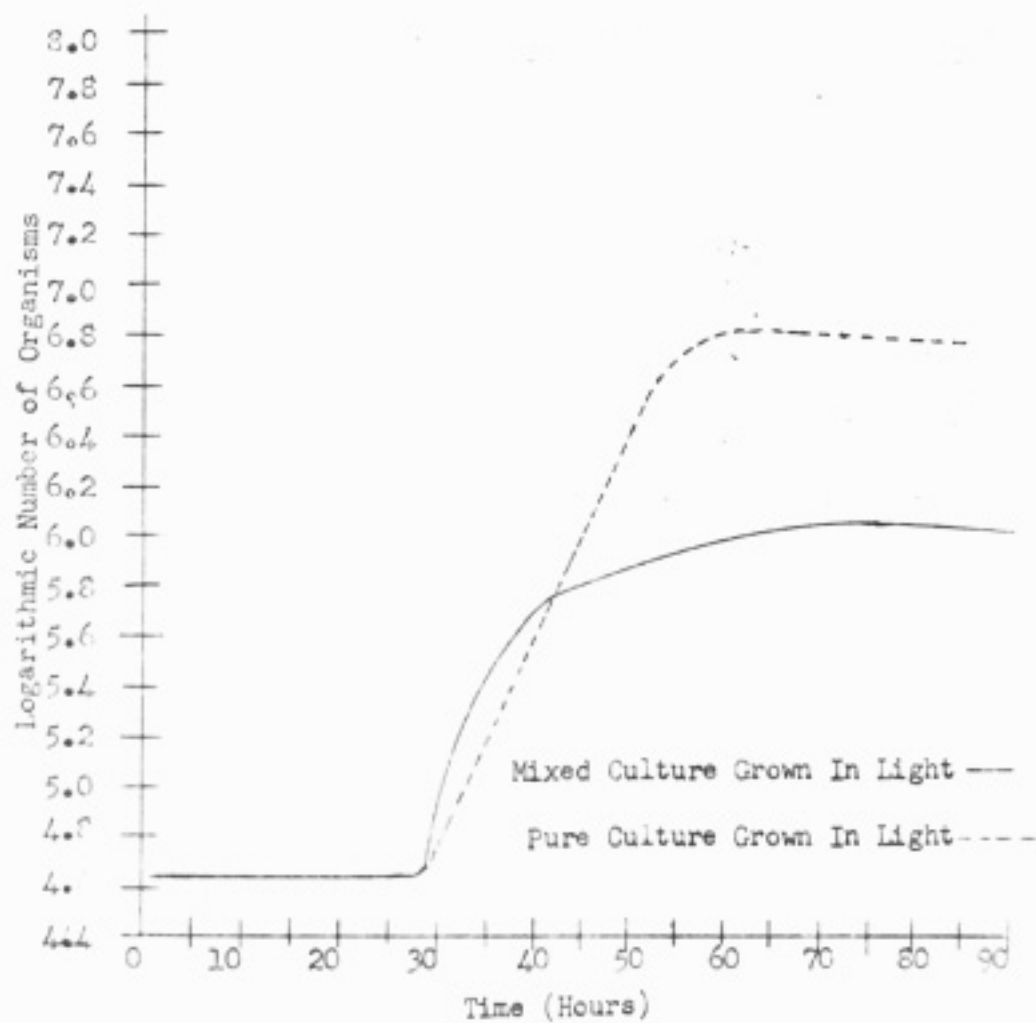


Figure 1. Growth of Rhodospirillum rubrum in light in pure and mixed culture.

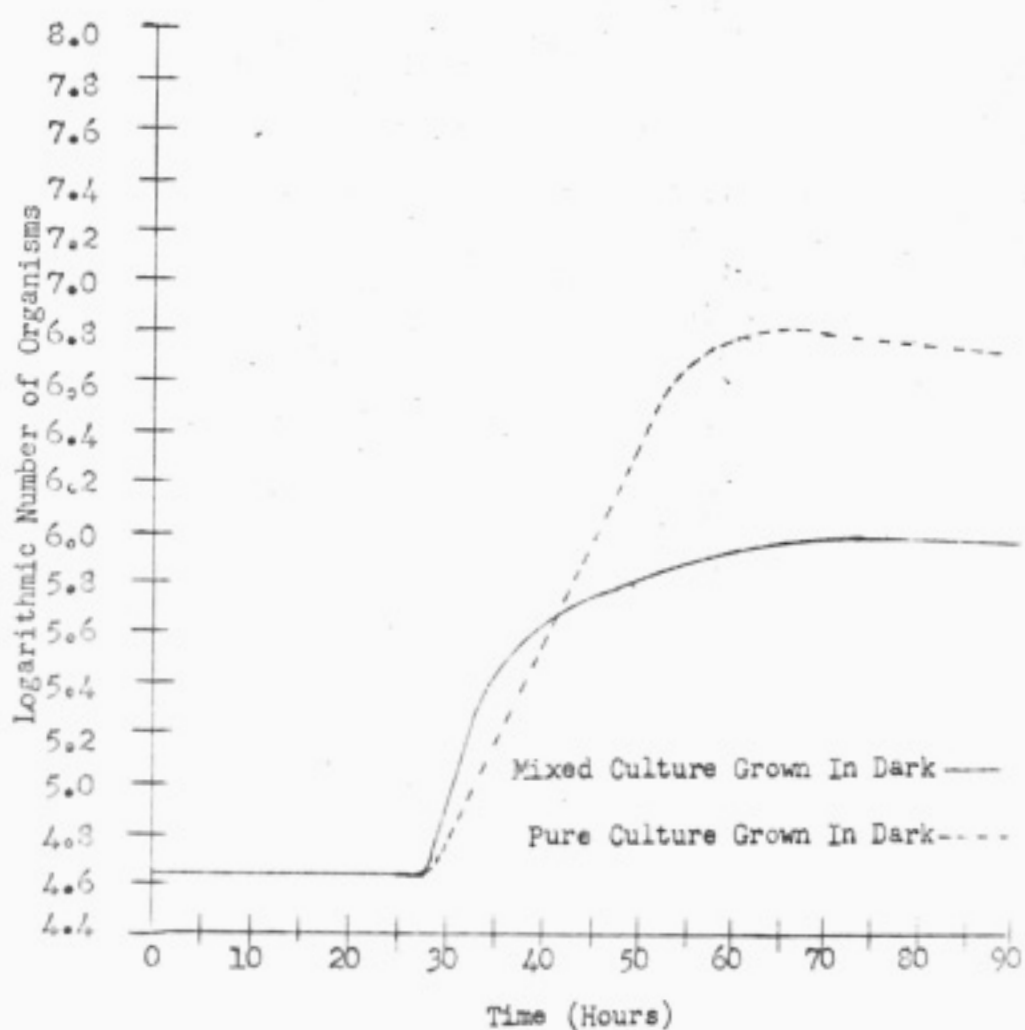


Figure 2. Growth of *Rhodospirillum rubrum* in dark in pure and mixed culture.



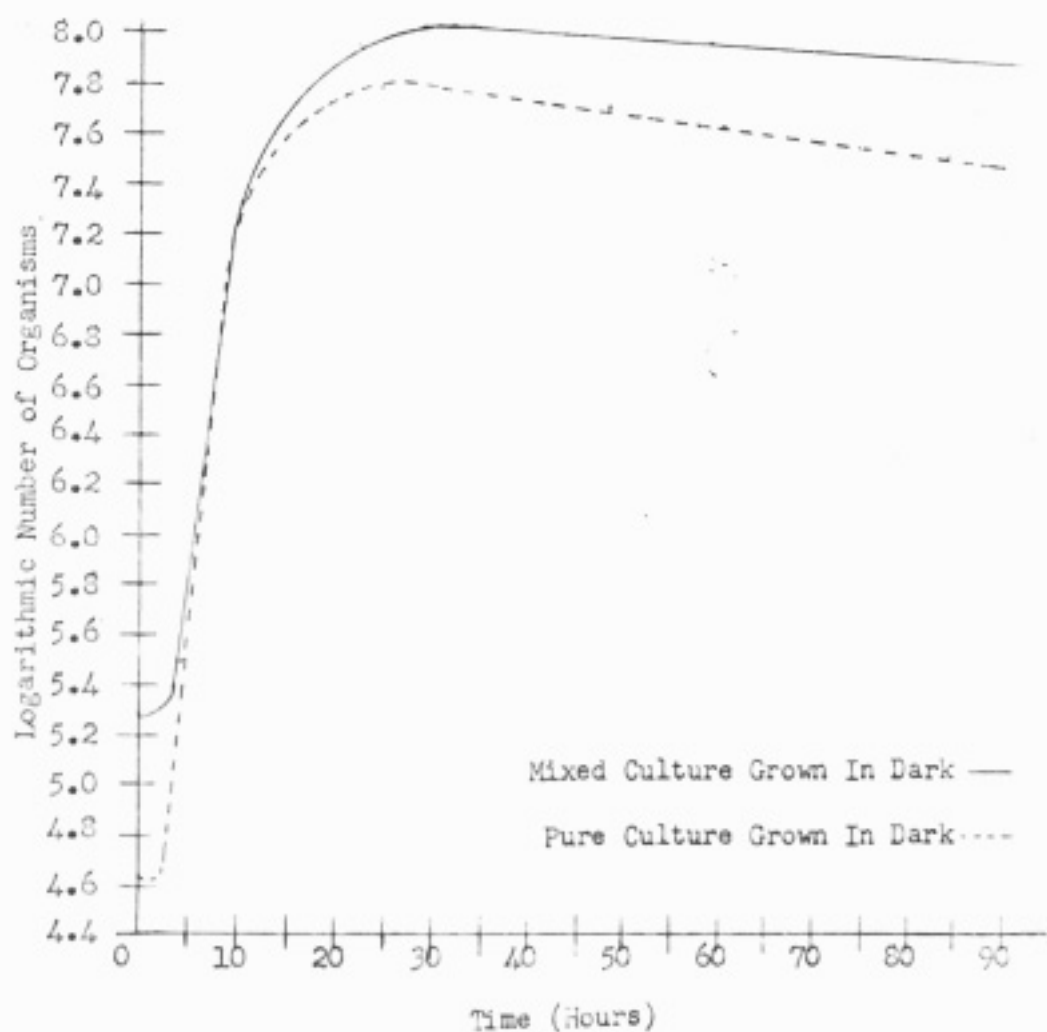


Figure 3. Growth of *Photobacterium fischeri* in dark in pure and mixed culture.

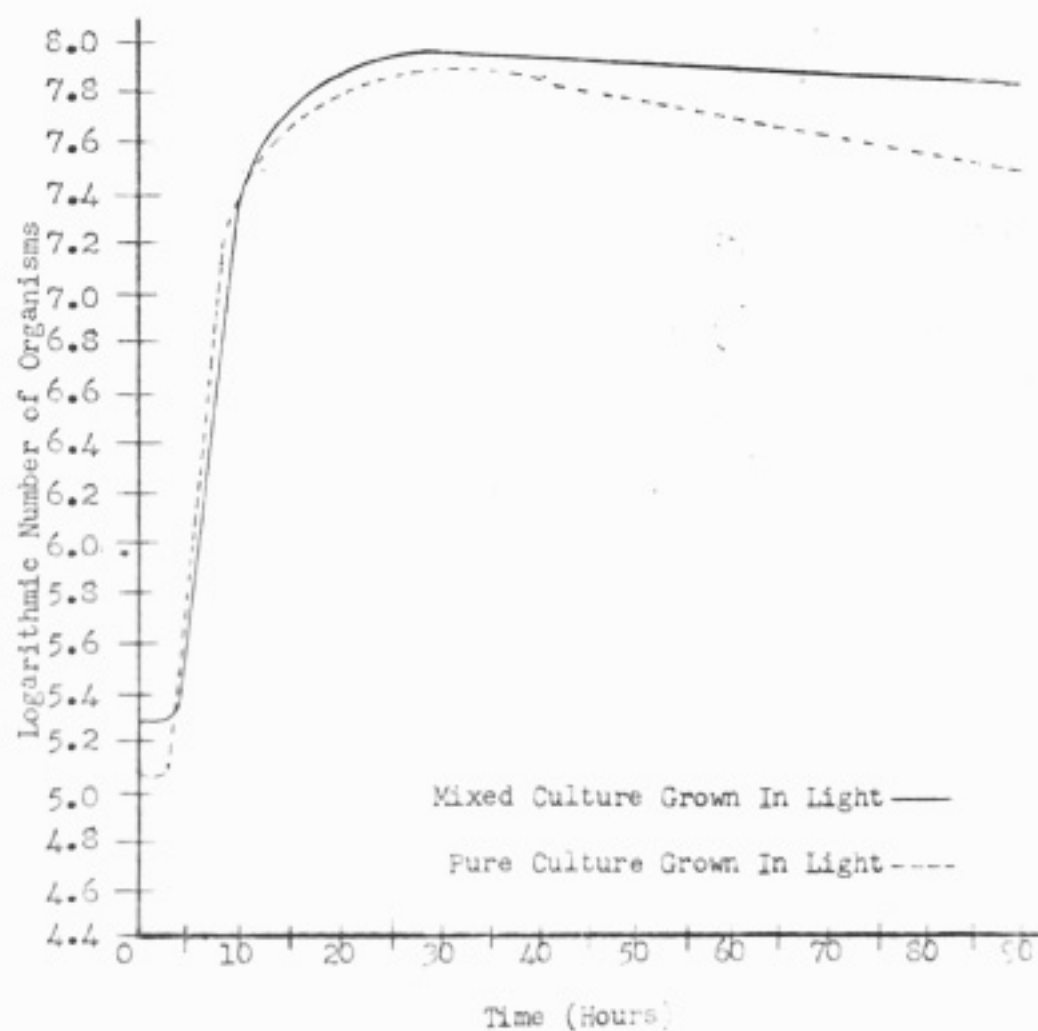


Figure 4. Growth of *Photobacterium fischeri* in light in pure and mixed culture.

TABLE I

GENERATION TIME IN HOURS OF RHODOSPIRILLUM RUBRUM GROWN IN  
PURE AND MIXED CULTURE IN TOTAL LIGHT AND DARK

Age of Culture in Hours	Pure Culture		Mixed Culture	
	Growth in Dark	Growth in Light	Growth in Dark	Growth in Light
25-30	4.13	4.13	1.66	1.66
30-40	1.28	1.28	2.83	2.76
40-50	1.48	1.48	7.81	7.46
50-60	3.71	4.36	9.40	8.15
60-70	9.58	9.40	7.81	8.15
70-90	20.40	20.40	17.10	17.80

TABLE II

GENERATION TIME IN HOURS OF PHOTOBACTERIUM FISCHERI GROWN IN  
PURE AND MIXED CULTURE IN TOTAL LIGHT AND DARK

Age of Culture in Hours	Pure Culture		Mixed Culture	
	Growth in Dark	Growth in Light	Growth in Dark	Growth in Light
00-04	00.54	01.14	01.24	01.24
04-08	00.22	00.24	00.61	00.61
08-12	01.55	01.55	00.55	00.45
12-24	05.75	06.04	05.00	06.04
24-36	12.04	12.04	11.50	11.50
36-60	24.01	24.60	24.20	24.50
60-84	23.99	24.95	24.15	24.15

TABLE III

GROWTH PHASES IN HOURS OF RHODOSPIRILLUM RUBRUM GROWN IN  
PURE AND MIXED CULTURE IN TOTAL LIGHT AND DARK

	Pure Culture		Mixed Culture	
	Growth in Dark	Growth in Light	Growth in Dark	Growth in Light
Lag Phase	27	26.5	27	26
Accelerated Phase	02	01.5	01	01
Logarithmic Phase	27	28.0	52	54
Stationary Phase	34	34.0	10	09

TABLE IV

GROWTH PHASES IN HOURS OF PHOTOBACTERIUM FISCHERI GROWN IN  
PURE AND MIXED CULTURE IN TOTAL LIGHT AND DARK

	Pure Culture		Mixed Culture	
	Growth in Dark	Growth in Light	Growth in Dark	Growth in Light
Lag Phase	00	00	00	00
Accelerated Phase	02	02	02	02
Logarithmic Phase	17	16	16	14
Stationary Phase	70	71	71	73

mixed culture increased to 7.81 hours for growth in dark and 7.46 hours for growth in light.

From fifty to sixty hours a continued increase in generation time was seen for the mixed culture which averaged 9.40 hours for growth in dark and 8.15 hours for growth in light. A subsequent increase in generation time also was seen for Rhodospirillum rubrum grown in pure culture. Rhodospirillum rubrum grown in pure culture for fifty to sixty hours showed an increased generation time to 3.71 hours for organisms in the dark and 4.36 hours for organisms grown in light.

Hours sixty to seventy had similar generation times for Rhodospirillum rubrum grown in mixed and pure culture. Organisms grown in pure culture showed an increase from sixty to seventy hours in generation time to 9.58 hours for organisms grown in dark and 9.40 hours for organisms grown in light. Rhodospirillum rubrum grown in pure culture from seventy to ninety hours showed a generation time of 20.40 hours, while organisms grown in mixed culture showed a generation time of 17.10 hours.

Table II, page 22, shows the generation times of Photobacterium fischeri at varying times in pure and mixed culture in total light and dark. The generation times from zero to four hours for pure culture in the dark were 0.54 hours and in the light 1.14 hours, while mixed cultures

showed generation times of 1.24 hours for organisms grown in dark and light. The large variation for Photobacterium fischeri grown in pure and mixed culture in dark can be seen by reference to Figures 3 and 4, pages 20 and 21. It can be noted that the inoculum of Photobacterium fischeri used for growth in pure culture in the dark was smaller than the inoculum used for the growth of pure culture in the light and of mixed culture in light and dark.

The generation times from four to eight hours were shortened for organisms grown in pure culture, being 0.22 hours for organisms grown in dark and 0.24 hours for organisms grown in light. Organisms grown in mixed culture showed a generation time of 0.61 hours for growth both in light and dark. A difference was seen for the generation times of Photobacterium fischeri grown from eight to twelve hours. Organisms grown in mixed culture showed a generation time of 0.61 hours for growth both in light and dark. A difference was seen for the generation times of Photobacterium fischeri grown from eight to twelve hours. Organism grown in pure culture showed a generation time of 1.55 hours for organisms grown in light. This difference can be observed by reference to Figures 3 and 4, pages 20 and 21, and Table IV, page 23, noting that the logarithmic phase of Photobacterium fischeri grown in mixed culture lasted for a longer time period than organisms grown in pure culture.

Photobacterium fischeri grown from twelve to twenty-four hours showed an increase in generation time to 5.57 hours when grown in the dark in pure culture and 6.04 hours when grown in the light in both mixed and pure cultures. A generation time of 5.00 hours was observed for organisms grown in mixed culture in dark. The generation times for Photobacterium fischeri grown from twenty-four to thirty-six hours were 12.04 hours for organisms grown in pure culture and 11.50 hours for organisms grown in mixed culture. Organism grown from thirty-six hours to sixty hours showed similar generation times for organisms grown in pure and mixed cultures. Photobacterium fischeri grown in pure culture showed a generation time of 24.01 hours in the dark and 24.60 hours in the light, while organisms grown in mixed culture showed generation times of 24.20 hours for organisms grown in dark and 24.50 hours grown in light.

The generation times for Photobacterium fischeri grown from sixty to eighty hours were very similar to the generation times for organisms grown from thirty-six to sixty hours. Organisms grown from sixty to eighty-four hours showed, in pure culture, generation times of 23.99 hours for organisms grown in dark and 24.95 hours for organisms grown in light. The generation times of Photobacterium fischeri grown in mixed culture were 24.15 hours in both light and dark.

Data from Figures 1 and 2, pages 18 and 19, are compared in Table III, page 23, which shows the growth phases of Rhodospirillum rubrum grown in pure and mixed culture in light and dark. A lag phase of twenty-seven hours was noted for the pure culture grown in dark and 25.50 hours for Rhodospirillum rubrum grown in light. Also a lag phase of twenty-seven hours occurred for organisms grown in dark in mixed culture and twenty-six hours for organisms grown in pure culture.

The accelerated growth phase extended over a longer time for organisms grown in pure culture than for organisms grown in mixed culture. Rhodospirillum rubrum grown in pure culture had an accelerated phase of 2.00 hours for organisms grown in dark and 1.50 hours for organisms grown in light, while organisms grown in pure culture showed an accelerated growth phase of one hour in both dark and light.

Logarithmic growth of Rhodospirillum rubrum continued for almost twice the time in mixed culture as in pure culture. In mixed culture logarithmic growth persisted for fifty-two hours in dark and fifty-four hours in light, while the logarithmic phase for the pure culture was noted for twenty-seven hours in dark and twenty-eight hours in light. A reverse is seen for the stationary phase when compared to the logarithmic phase. Stationary phases for Rhodospirillum rubrum were determined only to ninety hours, since bacterial



counts were not made beyond this time. The pure culture remained in a stationary phase for greater than thirty-four hours in both light and dark, and the mixed culture remained in the stationary phase for greater than ten hours in dark and nine hours in light.

Table IV, page 23, shows a comparison of the growth phases of Photobacterium fischeri in pure and mixed culture in total light and dark. No lag phase was found for organisms grown either in pure or mixed culture. An accelerated phase of two hours was recorded for Photobacterium fischeri grown in both pure and mixed cultures. Logarithmic phases of seventeen hours were recorded for Photobacterium fischeri grown in dark in pure culture and sixteen hours when grown in light. Similar readings of sixteen hours were recorded for organisms grown in mixed culture in dark and fourteen hours when grown in light.

The stationary phases for organism grown in pure and mixed culture was determined for growth to only ninety hours (since bacterial counts were not recorded exceeding ninety hours). A stationary phase greater than seventy hours was noted for Photobacterium fischeri grown in the dark in pure culture and greater than seventy-one hours when grown in the dark. A similar recording of greater than seventy-one hours was recorded for organisms grown in mixed culture in dark and greater than seventy-three hours for organisms grown in light.

It is shown by Figures 1 and 2, pages 18 and 19, that the growth of Rhodospirillum rubrum in mixed culture with Photobacterium fischeri is reduced when compared to growth in pure culture in Photobacterium broth. Several factors may be suggested to explain the decrease in the growth of Rhodospirillum rubrum in mixed culture. Competition or inhibition between Photobacterium fischeri and Rhodospirillum rubrum may have caused the reduced growth of Rhodospirillum rubrum in mixed culture. From the data in this investigation it has not been determined whether competition or inhibition occurred.

If growth inhibition was caused by Photobacterium fischeri, it might be determined by growing the bacteria in pure culture for a given period of time, filtering the bacteria from the Photobacterium broth, and then inoculating the Photobacterium broth with Rhodospirillum rubrum. If Rhodospirillum rubrum growth was inhibited, as it was when grown in mixed culture with Photobacterium fischeri, it could then be concluded that Rhodospirillum rubrum was inhibited by the growth of Photobacterium fischeri.

Rhodospirillum rubrum grown in pure culture in Photobacterium broth produced a sigmoid growth curve; however, Rhodospirillum rubrum grown in mixed culture showed a variation from the growth of Rhodospirillum rubrum in pure culture. It can be determined, however, that Rhodospirillum

rubrum, between twenty-five and thirty hours after inoculation in mixed culture, grew at a more rapid rate than Rhodospirillum in pure culture. It might be inferred that inoculation of Photobacterium fischeri into a culture containing Rhodospirillum rubrum enhanced the growth of Rhodospirillum rubrum for a period of time. It is suggested that Photobacterium fischeri may have produced a metabolic product which enhanced the growth of Rhodospirillum rubrum, or Photobacterium fischeri used certain compounds from the inoculum which enabled the Rhodospirillum rubrum to grow at a more rapid rate.

Photobacterium fischeri grown in mixed and pure culture varied little between the mixed and pure cultures. Photobacterium fischeri growth in mixed culture reached a greater maximal population than did the pure culture as can be seen by Figures 3 and 4, pages 20 and 21. This effect may have been caused by certain products formed by Rhodospirillum rubrum or by the formation of an inhibiting substance in the medium which the Rhodospirillum rubrum utilized in their metabolism.

A further investigation might be carried out by using an organism which reproduces only under photosynthetic conditions. It would then be possible to determine if there is sufficient light emitted by Photobacterium fischeri to induce photosynthesis.

## CHAPTER V

### SUMMARY

The present investigation has considered the growth relationship of Photobacterium fischeri and Rhodospirillum rubrum when grown in pure and mixed culture in total light and dark. A very slight difference was found between organism grown in light and dark. Both Rhodospirillum rubrum and Photobacterium fischeri utilized aerobic metabolism. Rhodospirillum rubrum has the potential to grow by photosynthesis; however, in this investigation it appears that some of the conditions required for photosynthesis by this organism were lacking.

A definite variation was found for Rhodospirillum rubrum when grown in pure and mixed culture. Rhodospirillum rubrum grown in pure culture produced a sigmoid logarithmic curve, while Rhodospirillum rubrum grown in mixed culture decreased in maximal population and produced a more variable growth curve.

Photobacterium fischeri grown in pure and mixed culture showed similar growth rates; however, Photobacterium fischeri grown in mixed culture had a maximal population greater than Photobacterium fischeri grown in pure culture.

It is suggested in further investigations of this type, an organism which may reproduce only under photosynthetic

conditions might well be used. By doing so it would then be possible to determine if the light emitted by Photobacterium fischeri is great enough to facilitate photosynthesis by a photosynthetic organism.

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